REMARKS

Claims 40-42, 44-77, 79, and 80 are pending. Claims 45-70 and 72-77 are withdrawn from consideration. Claim 40-42, 44, 71, 79 and 80 have been examined. Claims 40, 71, and 80 are amended in this response. No new matter is added.

The claims are rejected as indefinite and as obvious.

Rejections under 35 USC § 112, second paragraph

Claims 40-42, 44, 71, and 79-80 are rejected as indefinite. The rejection is traversed to the extent it is applied to the claims as amended.

The Examiner considers the term "antibiotic derived surface" as used in claims 40 and 71 to be unclear. However, the claims do not state that the surface is "... *derived with* ..." [an] antibiotic, but in fact state that the surface is *"derivatized with"* [an] antibiotic. The person having ordinary skill in the art would understand from this that the surface comprises chemical groups that have been chemically modified (i.e., "derivatized") with a bleomycin antibiotic in order to change their functionality. The term "to derivatize" was standard in the art as of the application's priority date. Indeed, the application as filed includes many examples of surfaces that have been derivatized by the binding of antibiotics (see, e.g. pages 12 and 13 at paragraphs 1.1 and 1.2).

Claim 40 recites the feature "said immobilized antibiotic". The Examiner considers there to be insufficient antecedent basis for this feature in the claim. Claim 40 has been amended to delete the term "immobilized" from claim 40.

The Examiner additionally states that it is unclear whether the Ble fusion protein is recombinant or whether the lysate comprises the protein and then the Ble marker is fused to the

protein and has asked for further clarification. The international application as filed provides a definition of fusion proteins at page 4, last paragraph to page 5, line 2 as follows:

> A "fusion protein", as used herein, refers to a protein, which comprises a "tag" at the N and/or C terminus which binds to members of the bleomycin family of antibiotics. The fusion protein may be expressed as a fusion protein from a ble gene containing genetic construct or may be formed by either intein mediated splicing of, for example, two separate polypeptides or by the chemical ligation of two such peptides according to methods known in the art.

One of ordinary skill in the art will readily understand that the fusion protein comprises the Ble marker protein (i.e., a protein that binds to members of the bleomycin family of antibiotics) at its amino or carboxy terminus and also the other protein of which the expression and folding is being detected. Although recombinant fusions are included within the scope of the invention, the method does not require the tag to be linked to the protein as a recombinant protein. Chemical ligation is also effective. All that is required by the claim is that the surface is contacted with a lysate which comprises the protein linked to the Ble marker protein.

The Examiner indicates that it is unclear in claim 40 how protein folding can he detected by binding of the Ble fusion protein to the immobilized antibiotic. The artisan would understand that if the protein to which the Ble marker protein is fused is not correctly folded, it will form an insoluble aggregate which will prevent the Ble marker protein from binding to the antibiotic on the surface. This is explained in the passage at page 7, lines 13-18 of the specification:

> When, for example, the Sh ble gene is fused in tandem with a second gene of interest, thereby encoding a fusion protein, expression of the fusion protein also confers resistance to Zeocin. If the gene of interest encodes an insoluble protein or protein fragment, the Zeocin resistance-conferring phenotype is not observed since the fusion protein is insoluble and incapable of binding the antibiotic.

The specification further explains (page 4, lines 5-13) that:

> The principle that underlies these systems is the observation that protein folding and solubility are closely correlated since misfolded protein usually forms insoluble aggregates or are heavily proteolysed by the host cell. It is therefore assumed that if a protein is solubly expressed in an unproteolysed form, it is in its correctly folded form. If a fusion is made between one protein and another, the folding and solubility of one domain is linked to that of the other. This is shown in Fig 1 which illustrates the principle of a folding marker for assessing solubility of the gene X expression product. Only when the protein product of gene X is soluble is the phenotype of the fusion apparent.

Thus, if the protein to which the Ble tag is bound is not correctly folded, it will prevent the Ble tag from binding to the bleomycin on the support. Binding to the support is therefore indicative of correct protein binding, as recited in amended claim 40.

Although Applicants do not agree with the indefiniteness rejection as applied to claim 44, in the interests of expediting prosecution Applicants have amended claim 44 to insert the term "gene" after each of "Sh ble" and "Tn 5 ble".

Claim 71 is amended to delete the term "optionally".

The Examiner further states that it is unclear how claim 71 differs from claim 40. Amended claim 71 differs from claim 40 in that it includes a releasing step. The specification teaches how to release the ble tag from the antibiotic. For example, when bleomycin is present at high density on the surface, it is possible to elute specifically bound protein using a high concentration of free bleomycin, i.e., there is competition between bleomycin conjugated to the surface and bleomycin in solution for binding to the bleomycin binding site on the protein (page 13, lines 14-17). When bleomycin is present at low density on the surface, the Ble fusion protein will be bound with low affinity. In such embodiments, the Ble fusion protein can be eluted by washing under appropriate conditions, for example, using increased salt concentration, competition with free bleomycin (which can be present at a lower

concentration compared to embodiments in which bleomycin is present on the array at high density), the use of chaotropic methods (e.g. washing with urea), heat, etc. Release using these methods is described at page 14, line 3; page 14, last line to page 15, line 1; and page 15, lines 11-13.

Thus, the specification teaches that there are multiple ways in which the protein can be released and these differ depending on the surface density of bleomycin that is used. One of ordinary skill in the art would know how to release a particular Ble fusion protein from a particular array in view of the state of the prior art and teaching provided in the specification.

Claim 80 is separately rejected as indefinite. The Examiner considers it to be unclear why the Ble fusion protein would bind to the marker when it is believed that said Ble fusion protein should bind to the antibiotic. Further, since claim 80 does not recite a specific marker, the Examiner further alleges that it is unclear if the antibiotic labeled with any marker would allow for proper binding to said Ble fusion protein.

This claim has been amended to require ... "...detecting binding of the ble fusion protein to the labeled antibiotic by detecting said marker." (emphasis added). Applicants submit that the claim as amended is clear.

Applicants request reconsideration and withdrawal of the rejections for indefiniteness in view of the foregoing amendments and remarks.

Rejections under 35 5 U.S.C. § 103(a)

Claims 40-42, 44, 71, and 79 are rejected as being unpatentable over Thinakaran (US 2003/0022151) in view of Takagi et al., US Patent No. 4,610,962. Applicants traverse these

rejections to the extent the rejection is applied to the claims as amended on the grounds that the Examiner has failed to establish a prima facie case of obviousness.

A *prima facie* case of obviousness requires that "either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *See* MPEP 706.02(j) citing *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985). Knowledge of the disclosure provided by the instant application must be put aside when determining whether the claimed invention would have been obvious. *See* MPEP 2142. The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one ordinary skill in the art. *See* MPEP §2143.01, citing *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398, 416, 82 USPQ2d 1385, 1396 (2007). Furthermore, a statement that modifications of the prior art to meet the claimed invention would have been "well within the ordinary skill of the art at the time the claimed invention was made" because the references relied upon teach that all aspects of the claimed invention were individually known in the art is not sufficient to establish a *prima facie* case of obviousness without some objective reason to combine the teachings of the references. *See* MPEP §2143.01, citing *Ex parte Levengood*, 28 USPQ2d 1300 (Bd. Pat. App. & Inter. 1993).

There is no objective reason provided by the Thinakaran and Takag references, alone or in combination, that would lead the skilled artisan to combine these references, nor is there any evidence that the resultant combination of these reference would have been predictable. Moreover, these references fail to provide the skilled artisan with a reasonable expectation that the methods claimed herein would be successful. Any suggestion that the claimed methods

would have been obvious or that using binding of a fusion protein to immobilized antibiotic to indicate expression and folding of the protein would have been predictable is an improper application of hindsight based on Applicants' specification. Thus, for the reasons provided below Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness.

Claim 40, from which the remaining claims subject to the rejection depend, is drawn to a method of detecting protein expression and folding comprising contacting a surface derivatized with a bleomycin family antibiotic with a cellular lysate, and assessing binding of the fusion protein to the antibiotic. Binding of the fusion protein to the antibiotic indicates expression and folding of said protein. The invention in this way exploits the binding properties of a bleomycin resistance gene to bleomycin.

The combination of Thinakaran and Takagi fails to describe or suggest the claimed invention. Thinakaran is cited for disclosing a method for screening zeocin resistance in cells expressing a PSI chimeric polypeptide that includes a presinilin fused to a yellow fluorescent protein (YFP) and an Sh ble marker protein (Sh ble). The method of Thinakaran is described in detail at page 4, paragraph 0049, as follows:

> If a candidate substance increases the accumulation of the chimeric protein that comprises the antibiotic resistance gene product, a cell expressing this chimeric protein can now be selected in a medium containing higher antibiotic concentration than prior to interaction with the candidate substance. For example, the bleomycin resistance gene (ble) encodes a protein, the bleomycin resistance protein (Ble), which provides antibiotic resistance by binding stoichiometrically with bleomycin. Thus, by the methods of the present invention, a) transfection of a cell with the chimeric PS/Ble polypeptide; followed by b) contacting/exposing/administering a candidate substance that can change the level of PS accumulation; and c) selecting cells that have an increase in PS accumulation at a higher concentration of the antibiotic allows the identification of a candidate substance that increases the accumulation of PS

proteins. This also allows identification of candidate substances that lead to enhanced stabilization and/or hyperaccumulation of PS proteins.

Thus, the assay disclosed in Thinakaran is an *in vivo* cell viability assay (see page 22 [0252]) that exploits the antibiotic resistance properties of a bleomycin resistance gene (see e.g. page 4 [0049]). In contrast, the claimed in vitro screening method requires a cellular lysate. Moreover, although Thinakaran discloses that an antibiotic resistance gene confers antibiotic resistance by stoichiometrically binding to the antibiotic, the significance of this statement is solely that stability of the protein to which the resistance protein is fused may be determined by correlating the amount of expression of resistance protein with the concentration of antibiotic required to kill the cells. It in no way suggests using the binding of the Ble resistance protein to bleomycin in an affinity capture system using cellular lysates, which is required by the claims.

Moreover, contrary to what is stated by the Examiner, it is not clear where Thinakaran "discloses various types of assays wherein said molecule can be bound to a surface or said protein can be bound to a surface". The *in vitro* binding assays mentioned at page 9 [0105], referred to by the Examiner, look at the interaction between a candidate modulator and an unstable protein. This section is concerned solely at looking at the ability of candidate modulators to bind to a target molecule because, as is explained at paragraph [0105]:

> The ability of a modulator to bind to a target molecule in a specific fashion is strong evidence of a related biological effect such as its ability to interact with and/or stabilize an unstable protein".

Although Thinakaran mentions that the unstable protein may be labeled, there is no suggestion of labeling the protein with an antibiotic resistance gene. The *in vitro* binding assays disclosed in this section do not suggest the claimed invention in any way. Thus, the Examiner is

mistaken that the only difference between Thinakaran and the present invention is that Thinakaran does not explicitly teach that zeocin is immobilized onto a surface. There would have been no motivation to adapt Thinakaran's *in vivo* cell viability assay, which uses the antibiotic resistance properties of the bleomycin resistance gene, to the claimed *in vitro* solid support binding assay, which utilizes the binding properties of the bleomycin resistance gene.

Takagi does not overcome the deficiencies of Thinakaran. Takagi discloses a carrier comprising an assembly of regenerated cellulose fibers on which a physiologically active substance is immobilized (see *e.g.* abstract and col. 5, lines 57-58). Antibiotics are listed at column 2 as examples of substances which may be immobilized on a cellulose carrier. A laundry list of antibiotics is provided in the paragraph bridging columns 2 and 3, of which bleomycin is just one of the many antibiotics mentioned.

However, Takagi does not in any way suggest the use of a carrier to which antibiotic is bound for detecting protein expression and folding. The Examiner has referred to the passage at col. 7, lines 8-28, which describes the use of a carrier having various proteins bound to it to remove various noxious substances from body fluid in order to treat various diseases (see also col. 6, lines 29-33). This does not lead to the method of the present invention. Takagi explains that the carriers may be used as a chemical reaction catalyst, a specific absorbent for separation and purification, a material for clinical examination, a medical material, and so forth (see e.g. abstract and col. 5, lines 59-62). There is no suggestion of using the carrier to detect protein expression and folding. Neither is there any suggestion of using the carrier to bind to the tagged portion of a fusion protein, let alone to the Ble marker portion of a fusion protein.

Thus, there would have been no motivation at the priority date to modify the cell viability assay of Thinakaran to a solid surface binding assay as claimed in the present invention in view of the teaching of Takagi.

Applicants note the Examiner's comments at page 5, lines 14-17 of the Office action:

> It would be reasonable for one of ordinary skill to know that screening assays can be modified such that either the molecule or protein is bound to the surface since regardless of which compound is immobilized, the binding reaction will still be detected and able to be quantified.

This statement is incorrect insofar as it relates to the present invention. The presently claimed method will work as a screen for expression and folding only if the antibiotic is bound to the surface and the Ble-tagged protein being assayed for expression and folding is present in the lysate. It will not function if the components are reversed, i.e., binding of the Ble-tagged protein to the surface and probing with an antibiotic in solution would not work, not least because the concentration of Ble-tagged protein in the lysate would determine the binding capture efficiency to the surface. The concentration of protein in some cases would result in low capture rates. The on rate and off rate of capture for different proteins would vary. Any attempt at quantitation between different proteins would be unreliable.In contrast to the Examiner's comment in the paragraph bridging pages 5 and 6, the combination of Thinakaran and Takagi does not disclose or suggest the active steps of the claimed method, even if for a different purpose.

Claim 71, which requires purifying the ble fusion protein from a crude extract, is dependent on claim 40 and so is non-obvious over Thinakaran in view of Takagi for the reasons provided above.

With respect to claim 79, the Examiner states that mass spectrometry was well known in the art for quantifying proteins. However, Applicants note that this was well known only as a downstream tool for analyzing proteins once they had been eluted from a binding matrix. In contrast, claim 79 relates to the use of mass spectrometry to determine binding of said fusion protein to said immobilized antibiotic, *i.e.* whilst the fusion protein is bound to the surface. There is no suggestion in either Thinakaran or Takagi to use mass spectrometry to determine protein binding to a surface in this way.

Claim 80 is separately rejected as unpatentable over Thinakaran in view of Takagi and in view of Calmels et al., Molecular Pharmacology 44:1135-1141, 1993. The rejection is traversed.

Claim 80 is also dependent on claim 40 and so is not obvious over Thinakaran in view of Takagi for the reasons presented above. Calmels does not add anything to the teaching of Thinakaran and Takagi to suggest the present invention.

In view of the foregoing amendments and remarks, Applicants request reconsideration and withdrawal of the rejections for obviousness.

Applicants submit that the application is in condition for allowance and request an action for same. Please charge any fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Attorney Reference No. 27353-513-US1.

Respectfully submitted,

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